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T Cells and the Regulation of Herpes Simplex Virus Latency and Reactivation

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Herpes simplex virus (HSV), like all herpesviruses, is a formidable adversary with numerous strategies to evade the immune system. The fact that the virus is able to persist indefinitely in the host suggests that for most of the time the immune system is powerless to act. The anatomy of infection is a relatively simple process. HSV infects epithelial cells in the mucosa or skin, then enters peripheral nerve endings and travels intraaxonally to the sensory ganglia. It is in neurons that the virus establishes a latent infection, from which there may be a periodic reactivation. Here infectious virus reappears and travels back along nerve fibers to epidermal sites in skin where a new round of replication is initiated, referred to as a recurrent infection. During the primary infection, a strong immune response evolves composed of virus neutralizing antibodies and an antiviral CD4 and CD8 T cell response, which efficiently inhibits virus replication at mucosal sites and in the nervous system (1, 2).

Latency is the principal strategy used by the virus to evade immune defences and for persisting indefinitely in the host. However, other evasion strategies also exist during the primary infection and after reactivation and/or recurrence. By infecting the nervous system, the virus takes advantage of natural "anatomical barriers" to evade immune defences. These include intraaxonal transport of virus particles, which renders virus invisible to antibody and cell-mediated immune mechanisms, and the natural deficiency of MHC class I molecules on neurons, which limits the activity of cytotoxic T cells. During latency, there is a general shut down of viral gene expression apart from a set of unusual transcripts, termed latency-associated transcripts (LATs), which are localized to the nucleus and do not encode any viral proteins (3). The function of LATs has still to be resolved; however, recent evidence suggests they may play a role in cell survival by blocking apoptosis (4). Clearly, the adaptation of HSV to this survival strategy in neurons tends to make it refractory to immune intervention. Consequently, the main windows of opportunity for

the immune response are during the primary infection and after reactivation of the virus to produce recurrent infections.

To dissect the complexities of the host-virus relationship, animal models have proved invaluable. Using mouse models of HSV infection, it is possible to derive detailed mechanisms of host resistance in different anatomical compartments. As with many other virus infections, the initial stages of HSV infection are influenced by the activity of type I IFNs and NK cells, which serve to limit the spread of virus to the nervous system (5, 6). As the adaptive immune response evolves, there is a clear role for T cells in the resolution of the primary infection. Adoptive transfer experiments of primed T cells from local LNs indicate an important role for CD4 T cells in resolving cutaneous infections, probably mediated by recruitment and activation of macrophages (2). Macrophages are potent inhibitors of HSV infection, in which nitric oxide and TNF- α are thought to play key roles (7, 8).

A key objective of the primary immune response at mucosal and cutaneous sites is not only to interrupt the spread of virus into the nervous system, but also to stem the flow of virus reemerging from axons to infect other sites on the same dermatome. This means that even during the primary infection the nervous system can act as conduits to spread virus around different epidermal locations, in a way not dissimilar to a zosteriform reaction, characteristic of shingles lesions produced by varicella zoster virus, a closely related herpesvirus. An HSV zosteriform model has been developed in mice, and has been used to investigate the immunological mechanisms responsible for interrupting the spread of virus to and from the nervous system (9). Aside from the activities of CD4 and CD8 T cells as interrupters of virus spread, other powerful inhibitors of this process are neutralizing antibodies. The passive administration of mAbs against HSV glycoproteins before infection and up to 3 d after infection inhibits the zosteriform spread of the virus (10). This indicates that as the virus moves from one compartment to another, i.e., from nerve ending to epithelial cells or vice versa, it becomes a target for neutralizing antibodies. However, once infection of epithelial cells takes place, neutralizing antibodies are ineffective. In this in-

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stance, T cell immune responses prevail. One may envisage similar mechanisms occurring within the peripheral nervous system, i.e., in the sensory ganglia or in the root entry zone, between the peripheral nervous system and central nervous system.

The nature of the immune response to HSV in the nervous system is qualitatively different from that seen in the skin. Resolution of the primary infection in sensory ganglia involves a major input from CD8 T cells (11). Early work clearly identified that in mice lacking CD8 T cells clearance of virus from the nervous system was delayed and resulted in a loss of neurons from sensory ganglia (12). In intact mice, although there was clear evidence of productively infected neurons (as detected by late viral antigen expression), there was no apparent loss of neurons, indicating that CD8 T cells were able to reverse a normally lethal effect by "curing" the relevant cells. There are similarities here with curing hepatocytes of hepatitis B virus by cytotoxic T cells (13). A key antiviral mechanism in both these infections probably involves IFN- γ . Why curing and not killing infected neurons is mediated by CD8 T cells could be related to the levels of expression of MHC class I. The low levels of MHC class I expression on neurons may be insufficient to facilitate a cytolytic event. Clearly, a loss of neurons during these early stages of infection is not only bad news for the host, but must also be viewed as limiting potential sites of latency for the virus. The balance between the host and the virus in this process is central to the number of latently infected neurons established, which in turn is probably related to the frequency of reactivations and/or recurrences.

A feature of the immune response in the sensory ganglia is the persistence of CD8 and CD4 T cells for prolonged periods after the resolution of the acute infection (14, 15). An important question is why do T cells persist and what role do they play? An explanation is provided in this issue by the work of Liu et al. (16). They used a mouse ocular model of HSV infection to analyze the virological and immunological events occurring in the trigeminal ganglion during a primary and latent virus infection. The analysis of virus latency and/or reactivation involved explanting ganglia or cells dissociated from ganglia in tissue culture and monitoring after several days the appearance of infectious virus. To study the effect of antiviral CD8 T cells on this process, ganglionic cultures were set up at different times post infection and either left untreated or were treated with anti-CD8 antibodies to block the action of resident ganglionic T cells. The outcome was quite dramatic in that resident ganglionic CD8 T cells, taken early in the latent period of infection, were highly effective at inhibiting virus reactivation. In contrast, the anti-CD8 treated cultures showed an accelerated appearance of reactivating virus. Although the efficiency of this process declined in ganglionic cultures taken at later times after infection (>30 d), the appearance of reactivating virus was nevertheless delayed. It is remarkable that relatively small numbers of T cells isolated from ganglia can have this inhibitory activity when dispersed in vitro. This begs questions on the mechanism(s)

involved in suppressing reactivation. The assumption is that this will involve the local action of cytokines (IFN- γ , TNF- α , and IL-6, all of which are elevated during latency and reactivation), rather than cytolytic mechanisms (17, 18). The nature of the cytokines involved could be defined by blocking their activity in vitro, or by using T cells from cytokine gene knock out mice in the ganglionic culture system. Another alternative is to carry out these experiments directly in cytokine gene knock out mice.

The persistence of CD8 T cells in sensory ganglia argues in favor of there being some viral antigen expression that acts to retain and recruit these T cells. As noted above, the general dogma is that there is no viral protein expression during latency. However, the initial stages of neuronal infection is probably more complex than previously thought, and may involve different levels of virus gene expression in some neurons that form part of a population of latently infected cells (3). Contributing to this picture is the immune response, which can clearly modify viral gene expression through the local action of inflammatory cytokines. Using more sensitive detection techniques, it has been possible to identify limited transcription of immediate early genes (notably ICP4) and early genes (e.g., thymidine kinase) (19). ICP4 is a key regulator of early and late gene expression, and is also a target for CD8 T cell recognition (20). This gene product was observed by Liu et al. in the trigeminal ganglia up to a month after infection (16), and could be one reason why CD8 T cells remain localized. The presence of ICP4 and CD8 T cells in sensory ganglia may serve a dual regulatory role. On the one hand, ICP4 acts as a T cell antigen serving to localize and activate T cells, while the activated T cells prevent any further viral gene expression probably through the action of cytokines, e.g., IFN- γ .

If viral proteins such as ICP4 are important for localizing T cells, then which cells are important in presenting these antigens. The assumption is that neurons are the likely candidates as they can express elevated levels of MHC class I during the early stages of HSV infection in ganglia, although it is questionable whether this happens during the latent infection (21). However, satellite cells (cells of macrophage lineage that tend to surround neurons) are more logical presenters because they express both MHC class I and class II. These cells are also infected during the primary infection and after reactivation, and may be important cells in regulating the immune response to virus. In addition, macrophages persisting along with T cells could serve to present viral antigens. A key question that needs to be resolved is for how long are T cells retained in ganglia, and does this correlate with the presence of viral protein expression? Perhaps T cell numbers never recede in this model system. Does the same pattern occur in human sensory ganglia?

HSV reactivation can occur following a variety of external stimuli, including stress due to temperature changes, after exposure to UV light, and following axotomy. The net effect is perturbation of neuronal function resulting in virus gene expression and the production of new virus particles, which travel along axons to infect skin and mucosa. In hu-

mans, this can result in a recurrent disease, such as genital lesions or cold sores. However, in the mouse, recurrences are rarely seen, which could be accounted for by the highly effective T cell response residing in latently infected ganglia. If this is the case, then it will be interesting to identify the antigenic specificity of eluted ganglionic T cells, which may be important in defining candidate viral proteins for use as vaccines. Other issues that need to be addressed in the light of Liu et al.'s findings (16) relate to modifying HSV reactivation in vivo by treating mice with anti-CD8 antibodies. Previous attempts to precipitate HSV recurrences by depleting T cells in vivo have proved unsuccessful; however, such experiments are now worth repeating in light of this new evidence.

The observations by Liu et al. (16) should stimulate renewed interest into the basic immunological mechanisms under pinning HSV infection. It certainly raises important questions on the nature of viral gene expression during latency, and new insights in to T cell recognition and control of these processes.

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References

1. Daeshia, M., L.T. Feldman, and B.T. Rouse. 1998. Herpes simplex virus latency and the immune response. *Curr. Opin. Microbiol.* 1:430-435.
2. Preston, C.M. 2000. Repression of viral transcription during herpes simplex virus latency. *J. Gen. Virol.* 81:1-19.
3. Perng, G.C., C. Jones, J. Ciacci-Zanella, M. Stone, G. Henderson, A. Yukht, S.M. Slanina, F.M. Hofman, H. Ghiasi, A.B. Nesburn, et al. 2000. Virus-induced neuronal apoptosis blocked by the herpes simplex virus latency-associated transcript. *Science*. 287:1500-1503.
4. Leib, D.A., T.E. Harrison, K.M. Laslo, M.A. Machalek, N.J. Moorman, and H.W. Virgin. 1999. Interferons regulate the phenotype of wild-type and mutant herpes simplex viruses in vivo. *J. Exp. Med.* 189:663-672.
5. Tanigawa, M., J.E. Bigger, M.Y. Kanter, and S.S. Atherton. 2000. Natural killer cells prevent direct anterior-to-posterior spread of herpes simplex virus type 1 in the eye. *Invest. Ophthalmol. Vis. Sci.* 41:132-137.
6. Karupiah, G., Q.W. Xie, R.M. Buller, C. Nathan, C. Duarte, and J.D. MacMicking. 1993. Inhibition of viral replication by interferon-gamma-induced nitric oxide synthase. *Science*. 261:1445-1448.
7. Kodukula, P., T. Liu, N.V. Rooijen, M.J. Jager, and R.L. Hendricks. 1999. Macrophage control of herpes simplex virus type 1 replication in the peripheral nervous system. *J. Immunol.* 162:2895-2905.
8. Simmons, A., and A.A. Nash. 1984. Zosteriform spread of herpes simplex virus as a model of recrudescence and its use to investigate the role of immune cells in prevention of recurrent disease. *J. Virol.* 52:816-821.
9. Simmons, A., and A.A. Nash. 1985. Role of antibody in primary and recurrent herpes simplex virus infection. *J. Virol.* 53:944-948.
10. Nash, A.A., A. Jayasuriya, J. Phelan, S.P. Cobbold, H. Waldmann, and T. Prospero. 1987. Different roles for L3T4+ and Lyt 2+ T cell subsets in the control of an acute herpes simplex virus infection of the skin and nervous system. *J. Gen. Virol.* 68:825-833.
11. Simmons, A., and D.C. Tschärke. 1992. Anti-CD8 impairs clearance of herpes simplex virus from the nervous system: implications for the fate of virally infected neurons. *J. Exp. Med.* 175:1337-1344.
12. Guidotti, L.G., T. Ishikawa, M.V. Hobbs, B. Matzke, R. Schreiber, and F.V. Chisari. 1996. Intracellular inactivation of the hepatitis B virus by cytotoxic T lymphocytes. *Immunity*. 4:25-36.
13. Shimeld, C., J.L. Whiteland, S.M. Nicholls, E. Grinfeld, D.L. Easty, H. Gao, and T.J. Hill. 1995. Immune cell infiltration and persistence in the mouse trigeminal ganglion after infection of the cornea with herpes simplex virus type 1. *J. Neuroimmunol.* 61:7-16.
14. Liu, T., K.M. Khanna, X. Chen, D.J. Fink, and R.L. Hendricks. CD81 T cells can block herpes simplex virus type I (HSV-1) reactivation from latency in sensory neurons. *J. Exp. Med.* 191:1459-1466.
15. Shimeld, C., J.L. Whiteland, N.A. Williams, D.L. Easty, and T.J. Hill. 1997. Cytokine production in the nervous system of mice during acute and latent infection with herpes simplex virus type 1. *J. Gen. Virol.* 78:3317-3325.
16. Liu, T., Q. Tang, and R.L. Hendricks. 1996. Inflammatory infiltration of the trigeminal ganglion after herpes simplex virus type 1 corneal infection. *J. Virol.* 70:264-271.
17. Shimeld, C., D.L. Easty, and T.J. Hill. 1999. Reactivation of herpes simplex virus type 1 in the mouse trigeminal ganglion: an in vivo study of virus antigen and cytokines. *J. Virol.* 73:1767-1773.
18. Kramer, M.F., and D.M. Coen. 1995. Quantification of transcripts from the ICP4 and thymidine kinase genes in mouse ganglia latently infected with herpes simplex virus. *J. Virol.* 69:1389-1399.
19. Martin, S., X.X. Zhu, S.J. Silverstein, R.J. Courtney, F. Yao, F.J. Jenkins, and B.T. Rouse. 1990. Murine cytotoxic T lymphocytes specific for herpes simplex virus type 1 recognize the immediate early protein ICP4 but not ICP0. *J. Gen. Virol.* 71:2391-2399.
20. Pereira, R.A., and A. Simmons. 1999. Cell surface expression of H2 antigens on primary sensory neurons in response to acute but not latent herpes simplex virus infection in vivo. *J. Virol.* 73:6484-6489.
21. Nash, A.A., and P. Cambouropoulos. 1993. The immune response to herpes simplex virus. *Semin. Virol.* 4:181-186.